1981

The effects of isoproterenol on myocardial histone acetylation.

Donna Lynn Wakeman. Young
University of Windsor

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE EFFECTS OF ISOPROTERENOL ON MYOCARDIAL HISTONE ACETYLATION

by

© Donna Lynn Wakeman Young

A Thesis

submitted to the Faculty of Graduate Studies
through the Faculty of
Human Kinetics in Partial Fulfillment
of the requirements for the Degree
of Master of Human Kinetics at
The University of Windsor

Windsor, Ontario, Canada

1981

To my husband Bruce and all
my family with sincere appreciation.

ABSTRACT

THE EFFECTS OF ISOPROTERENOL ON
MYOCARDIAL HISTONE ACETYLATION

by

Donna Lynn Wakeman Young

The effect of a single dose of isoproterenol (ISO) on myocardial histone protein acetylation was studied in the isolated perfused rat heart. Female Wistar rats (200-220g) were treated with isoproterenol for 1, 2, and 3 hours. After sacrifice the hearts were removed, and perfused on a Langendorff apparatus for 5, 10, 20, 40, or 80 minutes with a Krebs-Henseleit bicarbonate buffer containing [^{3}H] acetate (2uCi.ml^{-1} buffer). Following perfusion, myocardial nuclei were isolated and the histones extracted and fractionated using urea polyacrylamide gel electrophoresis.

The data from this investigation do not show any statistically significant differences in acetylation between ISO treated animals and saline treated controls in the total histones isolated or in the electrophoretically purified histone fractions.

These data suggest that either; 1) the ISO administered was not effective as a stimulatory agent, or 2) the time period examined was not appropriate, or 3) using this

model, histone acetylation did not increase significantly, or 4) because of the small sample size used in this study, no differences were statistically significant, or 5) using this model, histones were primarily functioning as a protective mechanism for DNA.

ACKNOWLEDGEMENTS

The author is indebted to Dr. P. B. Taylor, Dr. G. W. Marino, and Dr. B. Virgo for their invaluable guidance and assistance throughout this investigation.

Special thanks are extended to Bruce Young for his technical assistance, and to Mr. J. Bolger of the Psychology Department for the animal donations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

The mammalian heart has the ability to adapt by hypertrophy to numerous environmental stresses exerted upon it. Compensatory hypertrophy is accomplished by biochemical and physiological adjustments of the cells in response to pathological or physiological stimuli (Fanburg, 1970; Wikman-Coffelt, et al., 1979). Since the mammalian myocardium loses its mitotic capacity shortly after birth, the subsequent growth of muscle tissue must be a consequence of hypertrophy of the pre-existing myocytes (Fanburg, 1970; Wikman-Coffelt, et al., 1979).

Several biochemical changes occur in response to an increased work load placed on the heart. In experimentally induced cardiac hypertrophy, there is a marked stimulation in RNA and protein synthesis (Rabinowitz, et al., 1970; Schrieber, et al., 1967; Fanburg, et al., 1968; Koide, et al., 1969; Nair, et al., 1968). Schrieber et al. (1967), have shown that one of the earliest responses to work overload was an increased RNA polymerase activity within four hours after aortic constriction. Subsequently, Fanburg (1970) reported a significant net increase in tissue RNA concentration between 24 and 48 hours of aortic constriction. Severe cardiac hypertrophy may be accompanied by a significant increase in total DNA content per heart. However, this has

been ascribed to alterations of DNA synthesis in non muscle cells (ie. connective tissue and endothelial cells) (Meerson, 1969; Rabinowitz, et al., 1970; Morkin, 1974; Cutilletta, et al., 1975, Morkin, et al., 1968).

Experimentally, several physical and chemical methods have been used to induce myocardial stress to produce cardiac hypertrophy (Cutilletta, et al., 1975; Fanburg, 1970; Fanburg, et al., 1968; Koide, et al., 1969; Meerson, 1969; Morkin, 1974; Morkin, et al., 1968; Nair, et al., 1968; Schrieber, et al., 1967; Wikmen-Coffelt, et al., 1979; Wood, et al., 1971.) Isoproterenol (ISO), a catecholamine synthetic, has been used by several investigators as a convenient and reliable method to stimulate cardiac muscle growth (Källfelt, et al., 1976; Stanton, et al., 1967; Stanton, et al., 1969; Taylor, et al., 1977; Wood, et al., 1971). The beta-adrenergic stimulating capacity of ISO can produce arterial hypotension and persistent tachycardia (Rona, et al., 1959; Stanton, et al., 1967; Stanton, et al., 1969). It is believed that the increased myocardial oxygen demands produced by this beta stimulator, coupled with a reduced systemic arterial blood pressure and therefore, reduced coronary perfusion pressure, results in temporary myocardial hypoxia and a shift from oxidative to glycolytic metabolism (Rona, et al., 1959; Stanton, et al., 1967; Stanton, et al., 1969; Takenka, et al., 1974).

Rona et al. (1959) was the first to describe the use of ISO to study the severity of cardiac necroses and subsequent repair following large and repeated doses. Several investigators have shown that repeated ISO administration at low concentrations produces cardiac hypertrophy (Stanton, et al., 1967; Stanton, et al., 1969; Taylor, et al., 1977; Wood, et al., 1971). Repeated subcutaneous injections of ISO (0.3mg.kg^{-1}.24 hr.$^{-1}$ for 12 consecutive days) produced a 40% increase in heart weight/body weight ratio and a 75% increase in left ventricular and septal muscle fiber area (Taylor, et al., 1977). Stanton et al. (1967) found that ISO (80mg.kg^{-1} body weight) administered twice daily for 2 consecutive days produced a significant increase in RNA content and RNA/DNA ratio. In a subsequent study, Stanton et al. (1969) reported similar changes in RNA synthesis and also an increase in the incorporation of C^{14} labelled amino acids following ISO treatment. Wood et al. (1971) produced a 300-400% increase in RNA synthesis 3 hours after a single subcutaneous injection of ISO (5mg.kg^{-1} body weight).

Since RNA synthesis appears to be one of the earliest responses to ISO stimulation, it seems reasonable to suggest that factors affecting RNA synthesis may be important in regulating this form of muscle growth. The three types of RNA involved in protein synthesis are transcribed from complimentary base sequences in DNA by the enzymes RNA polymerase

(I,II,III): Ordinarily, 80-90% of the nuclear DNA is repressed and is not actively utilized as a template for RNA synthesis (Ris, 1975). Derepression of the DNA (by some physiological or biochemical mechanism must either accompany or precede RNA synthesis and RNA polymerase activity, followed by subsequent protein synthesis.

The chromosomal material, or chromatin, in eukaryotic cells contains histones and non-histone proteins complexed with DNA in a repeating pattern. Histones, the most intensely studied nucleoproteins, have been classified into H1, H2A, H2B, H3, and H4, and described in detail (Delange, et al., 1975). Every 140-200 base pairs of DNA are complexed with a core of histones containing two molecules each of species H2A, H2B, H3, and H4. The DNA is wrapped externally around the core of histone proteins (Kornberg, 1977). Histone H1 is not attached to the nucleosome core, but appears to be attached to DNA in the linker region between nucleosomes. The close association of histones with DNA suggests they may be implicated in functions involving the control of the shape of the DNA. DNA depletion and reconstitution studies indicate that histones are involved in maintaining the folded structure of chromatin. The selective removal of histones from calf thymus nuclei resulted in a 300-400% increase in RNA synthesis. Conversely, the restoration of histones to the histone-depleted nuclei was followed by an immediate

decrease in RNA synthesis (Allfrey, et al., 1962). Bonner et al. (1968) have shown that the removal of histones by salt extractions with solutions of increasing molarity resulted in a stepwise increase in RNA synthesis.

Chemical modifications (ie. methylation, phosphorylation, and acetylation) of specific amino acid side chains within histones have been shown to alter the structure of the chromosome which may act as a derepressor of the genome by exposing segments of the DNA to serve as a template (Bradbury, 1975). Experiments involving the chemical acetylation of nuclear proteins causing modification of histone structure have shown changes in histone-DNA interactions and RNA polymerase activity (Allfrey, 1968). A physiological mechanism that appears to influence histone binding is enzymatically controlled acetylation. Smulson (1979) has reported that acetylation and phosphorylation of nuclear proteins are important in transcriptional regulation. A temporal relationship has been demonstrated between changes in histone acetylation and DNA template activity and RNA synthesis (Liew, et al., 1972). Pogo et al. (1966, 1968) have provided evidence suggesting that increased histone acetylation appears to preceed enhanced nuclear RNA synthesis in phytohemagglutinin treated lymphocytes and regenerating liver. Liew et al. (1972) reported a 230% increase in heart nuclei acetylation 40 minutes after aldosterone treatment of adrenalectomized rats, followed by increased RNA polymerase activity and

RNA synthesis.

It has been suggested by these and other investigators, that the post-synthetic covalent modification of the basic nuclear proteins (histones) may be a preliminary step in the activation of DNA. In the present study, a single injection of ISO was used as a pharmacological stimulus and the response of myocardial histone acetylation was investigated.

CHAPTER II

METHODS

Animal Care and Handling

Female Wistar rats weighing 200-220 grams, were housed in pairs and provided Purina laboratory chow and water ad libitum. All animals were fasted overnight before use.

Isoproterenol Administration

Isoproterenol hydrochloride (5mg.kg^{-1} body weight, and 25mg.kg^{-1} body weight) solubilized in saline was injected subcutaneously and allowed to circulate for 1, 2, or 3 hours. Control animals received subcutaneous injections of saline 1 hour prior to sacrifice.

Beta Blockade

Propranolol hydrochloride (25mg.kg^{-1} body weight) was solubilized in saline and injected subcutaneously 2 hours prior to sacrifice.

Method of Perfusion

Following the appropriate treatment, the animals were anesthetized with sodium pentobarbital (Nembutal 30mg i.p.). A mid-line ventral incision was made with scissors to open the abdominal cavity. Two hundred units of heparin

was injected into the inferior vena cava and allowed to ciruclate for 30 seconds to prevent blood clotting. The diaphragm was transected and the heart removed and quickly placed into ice cold saline to arrest contractions. The heart was then cannulated by the aorta on a Langendorff perfused heart system (Morgan, et al., 1961). The hearts were perfused with a Krebs-Henseleit bicarbonate buffer containing 15mM glucose (Krebs, et al., 1932). The perfusate was maintained at 37°C and gassed with a 95%O_2:5%CO_2 mixture saturated with water. Following a 10 minute preliminary wash out perfusion to remove the blood, the heart was transferred to a recirculating system containing 30ml of perfusate and [^{3}H] sodium acetate, 2uCi.ml^{-1} buffer (specific activity 2.0Ci.mmole^{-1}). The first 5ml of buffer was collected and discarded to reduce tissue dilution and the remaining 25ml recirculated for variable times at a perfusion pressure of 60mmHg. Following the perfusion, the heart was cut from the cannula, wrapped in tin foil, quickly frozen in liquid nitrogen, and stored at -40°C.

Isolation of Nuclei

Two perfused hearts were pooled and the nuclei isolated by the method of Liew et al. (1973). All procedures were carried out at 0-4°C. The tissue was thawed, trimmed of the great vessels and atria, blotted, and weighed. The hearts were minced with scissors and homgenized in a Potter

Elvejem tissue homgenizer in 2 volumes of solution A containing 10mM Tris-HCl (pH 7.2), 1mM $MgCl_2$, 0.25M sucrose, and 0.1M phenylmethlysulfonylflouride (PMSF), as a protease inhibitor, and centrifuged at 3000 rpm for 10 minutes. The pellet was rehomogenized in solution A, filtered through 2 layers of fine nylon, and centrifuged at 3000 rpm for 10 minutes. The pellet was suspended in solution B containing 10mM Tris-HCl (pH 7.2), 1mM $MgCl_2$, 0.25M sucrose, 0.1M PMSF, and 0.1% Triton X-100, and centrifuged at 3000 rpm for 10 minutes. The pellet was suspended in solution C containing 10mM Tris-HCl (pH 7.2), 1mM $MgCl_2$, 2.2M sucrose, and 0.1M PMSF underlayed with 5ml of solution C and centrifuged at 25,000 rpm for 60 minutes in a Spinco SW 25.1 rotor. The supernatent was removed from the centrifuge tubes by inversion and the walls gently rinsed with solution A and wiped dry.

Extraction of Histone Proteins

The histone proteins were extracted by methods previously described (Liew et al., 1973). The nuclear pellet was suspended in solution A, and centrifuged at 3000 rpm for 10 minutes. The pellet was washed twice with solution D containing 10mM Tris-HCl (pH 7.2), 0.14M NaCl, and 0.1M PMSF and centrifuged at 10,000 rpm for 10 minutes. The pellet was extracted twice for 30 minutes each with 0.25N HCl and 0.1M PMSF at 0-4°C. The supernatents were

pooled and the histones precipitated overnight at 4°C in ten volumes of ice cold acetone. The precipitate was recovered by centrifugation, dried and solubilized in 300ul of 0.01N HCl in 15% (w/v) sucrose.

Electrophoretic Fractionation of Histones

Histones were fractionated electrophoretically by the method of Panyim and Chalkley (1969). A 60ug sample was layered on a 9cm, 6.25M urea polyacrylamide gel, and electrophoresed at 1.75mA.gel^{-1} for 4.5 hours. The gels were removed and stained overnight in 0.1% Amido Black in 7% acetic acid and destained by diffusion in 7% acetic acid.

Determination of Protein and Radioactivity in Histone Fractions

To determine the amount of protein and specific radioactivity in each histone fraction, duplicate gels were run for each sample. For protein content, the bands in one gel were sliced, quartered, and the colour eluted in 0.6ml of dimethyl sufoxide at 40°C for 72 hours. Optical densities were measured at 615nm as described by Wangh et al. (1972). The protein in each fraction was calculated from the percent of the total optical density units in each gel and expressed as ug protein per fraction.

In a second gel, each protein band was removed by slicing and the slices digested in 0.5ml of 30% H_2O_2 over-

night at 40°C. To reduce spurious results, the digest was dark adapted for 72 hours before counting. The radioactivity in each fraction was expressed as CPM.ug^{-1} protein.

Determination of Protein and Radioactivity

The protein yields were quantified by the method of Lowry et al. (1951) using calf thymus histone as standard.

A 50ul sample of the isolated histone was suspended in 10ml of toluene based scintillation solution containing 4gm PPO and 50mg POPOP per litre of toluene, and the radioactivity determined in a Beckman Liquid Scintillation Counter LS-100. The specific radioactivity in each sample was expressed as CPM.mg^{-1} protein.

Statistical Analysis

The data were analyzed by independent Student t-tests, and Wilcoxan Mann Whitney U tests. Statistical significance was accepted with a p-value of 0.05 or less.

CHAPTER III

RESULTS

The Effect of ISO Treatment on Histone Acetylation and Protein Yields

The effect of isoproterenol on total histone acetylation is shown in Table 1. Hearts were perfused for 60 minutes following treatments of 1, 2, and 3 hours with ISO (5mg.kg^{-1} body weight). Control hearts were perfused after similar volumes of saline injection. The results indicate that there was no significant difference in histone acetylation after 1, 2, or 3 hours of treatment. The protein yields expressed as ug.g^{-1} tissue wet weight also showed no significant difference. This suggests that lack of a difference is not the result of variable amounts of isolated histones since the protein yields were similar.

TABLE I

Effect of ISO treatment on Histone Acetylation and Protein Yields (N=3).

Saline Control	1 Hour ISO	2 Hours ISO	3 Hours ISO
CPM.mg^{-1} Protein			
5255 ± 1158	4742 ± 1139	5365 ± 1570	2856 ± 908
Yield ug.g^{-1} Tissue Wet Weight			
107.21 ± 9.69	111.75 ± 0.22	115.85 ± 6.57	104.52 ± 8.48

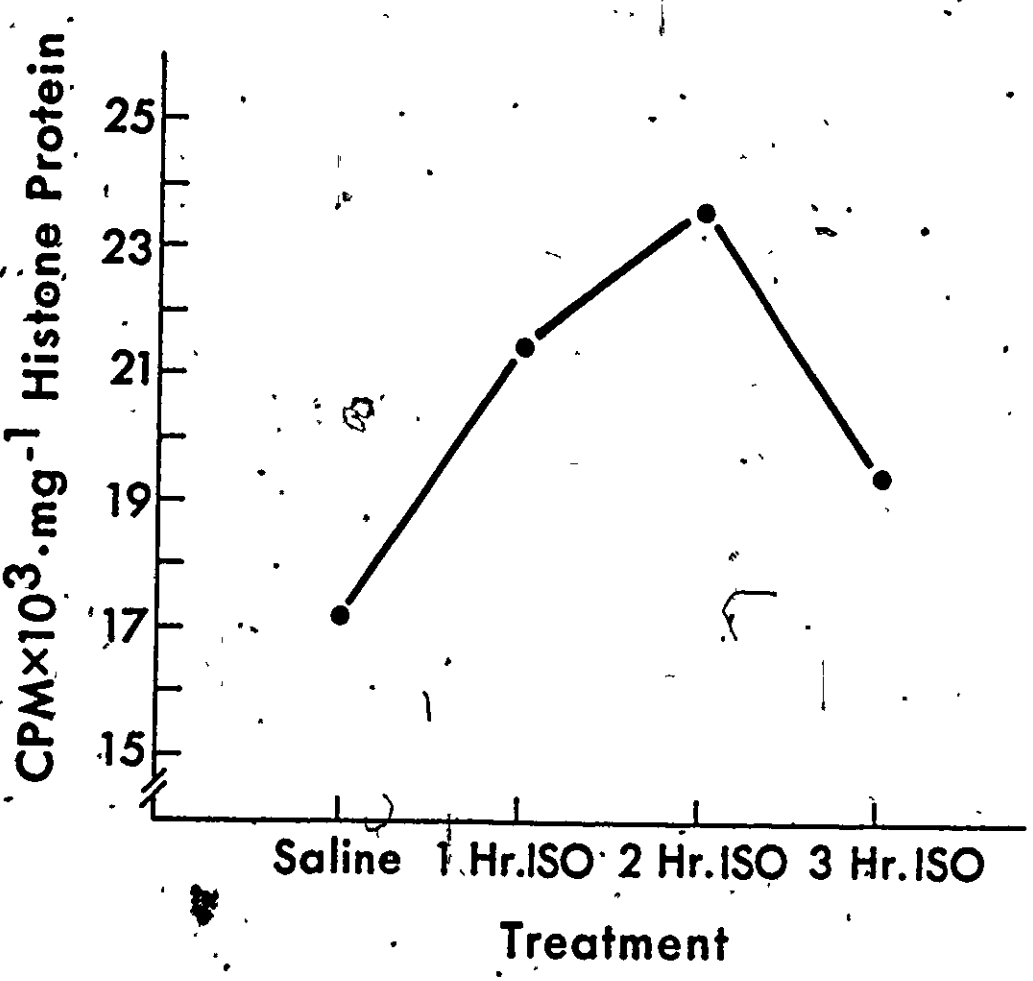
Values are means ± S.E.M.

The Effect of ISO Treatment Time on the Acetylation of Electrophoretically Purified Histones

The total histones were fractionated electrophoretically in urea-polyacrylamide gels. Duplicate gels were run for each sample and the fractions analyzed for protein content and radioactivity as described in the Method section. Four major fractions were identified and the incorporation expressed as $CPMx10^3.mg^{-1}$ protein. Figure 1 represents the total amount of radioactivity as determined by the sum of the four fractions of electrophoretically purified histones. These results revealed no significant difference in acetylation between the saline control and ISO treatments although there appeared to be an increase at 1 and 2 hours of ISO treatment. The mean of the saline controls was 17.2735 $CPMx10^3mg^{-1}$ protein compared to 21.4792 $CPMx10^3.mg^{-1}$ protein and 23.6968 $CPMx10^3.mg^{-1}$ protein for the 1 and 2 hour ISO treated animals respectively. This difference was not significant however when tested statistically.

Figure 2 represents the radioactivity in each fraction at various ISO treatment times. No differences were statistically significant although all four fractions appear to increase after 2 hours, and decrease at 3 hours. The fractions were not uniformly acetylated. Histone H3 was most highly acetylated, followed by fraction H4. The fractions containing H1 and H2 were progressively less acetylated.

FIGURE 1: The Effect of ISO Treatment Time on Total Radioactivity in Electrophoretically Purified Histones, determined by the sum of the four fractions at each condition. There are no significant differences as determined by independent student T-tests, and Wilcoxan Mann Whitney U tests. Each point represents the mean of 3 experiments.


CPM×10³.mg⁻¹ Histone Protein
25
23
21
19
17
15
Saline
1 Hr.ISO
2 Hr.ISO
3 Hr.ISO
Treatment

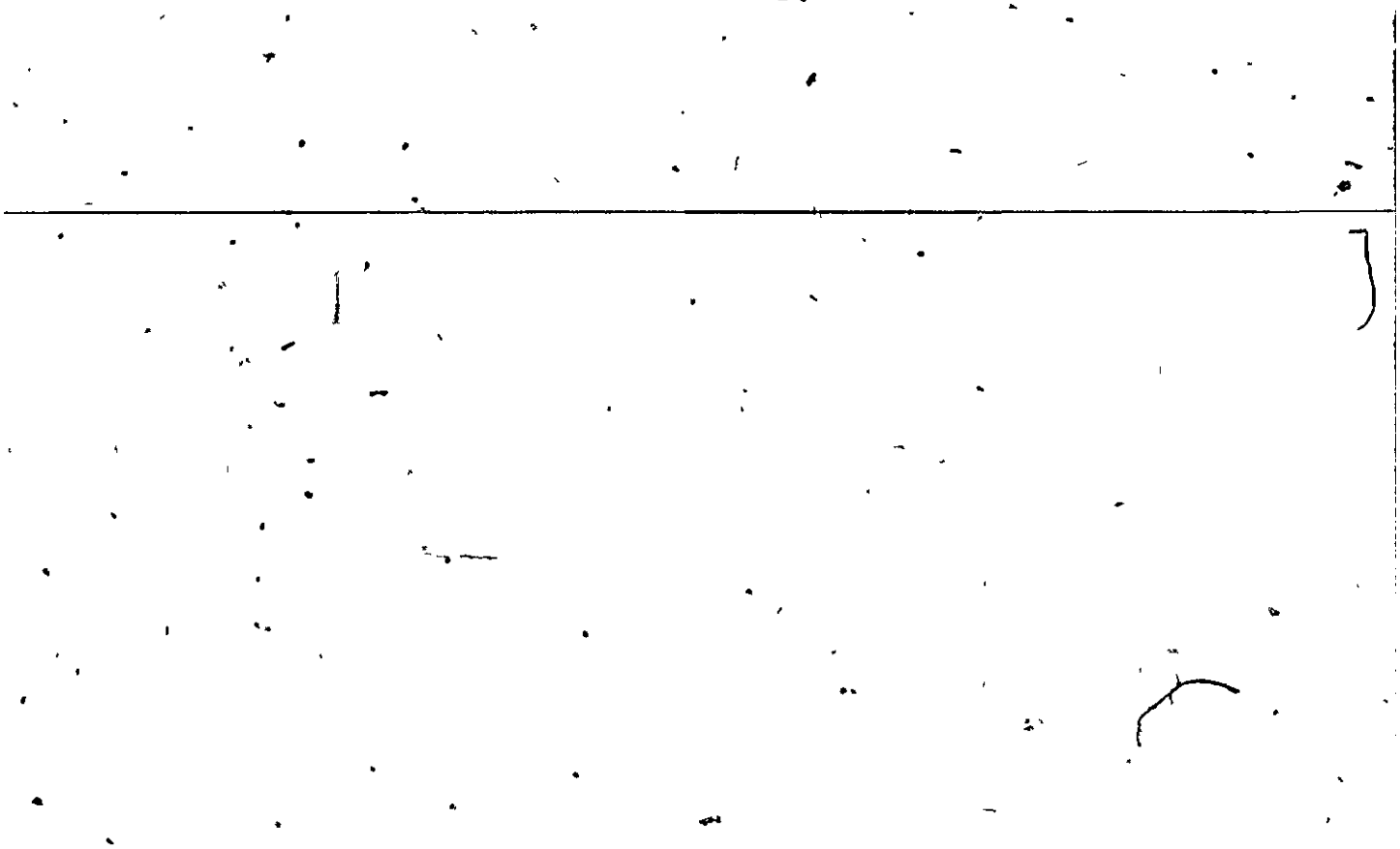
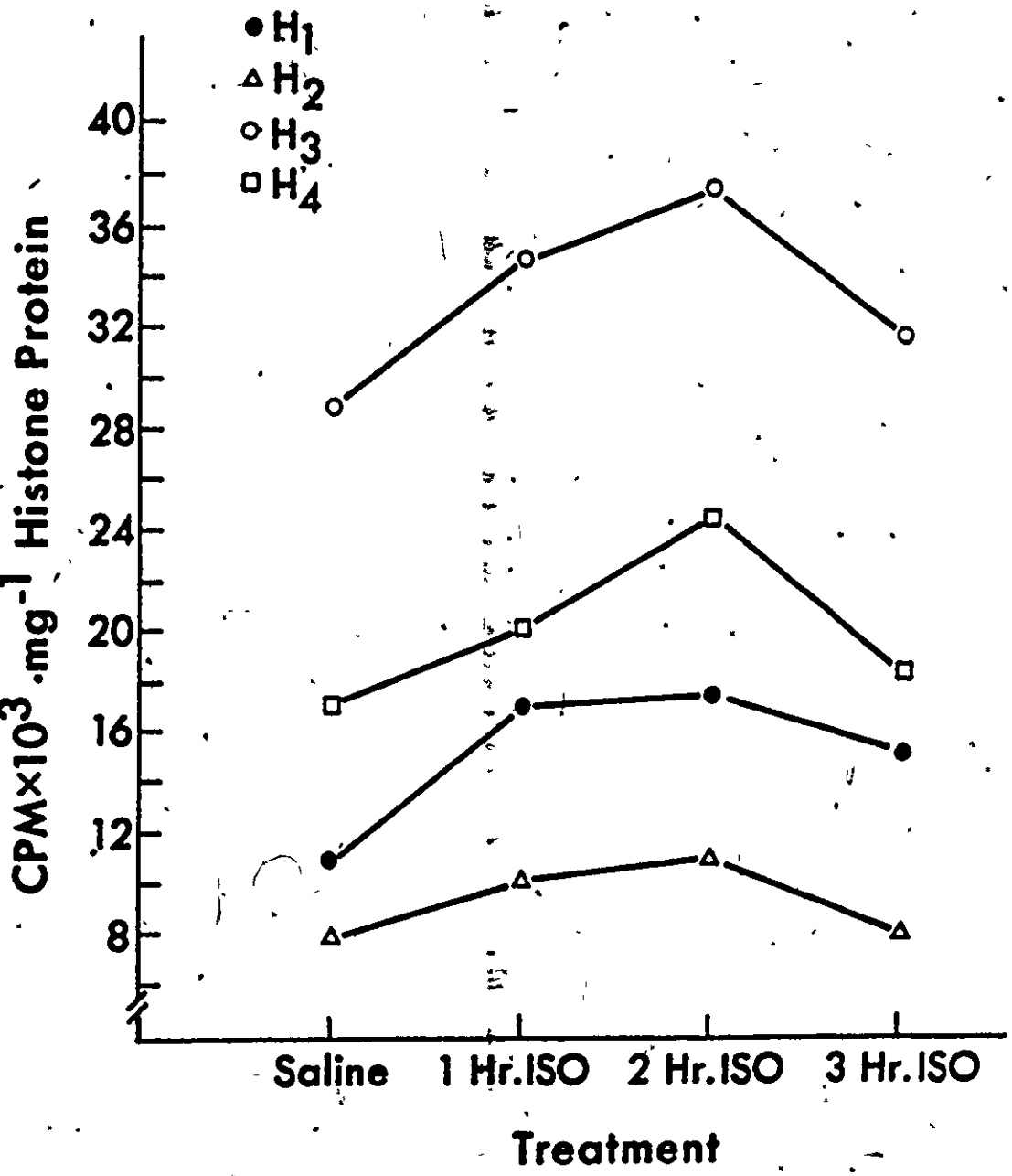


FIGURE 2: The Effect of ISO Treatment Time on Acetylation in Purified Histone Fractions. Histones isolated from [^{3}H] acetate labelled perfused hearts were fractionated electrophoretically on urea polyacrylamide gels. Radioactivity and protein content of each fraction were determined as described in the Method section. Each point represents the mean of 3 experiments.


● H_1
△ H_2
○ H_3
□ H_4
40
36
32
28
24
20
16
12
8
CPM×10³.mg⁻¹ Histone Protein
Saline
1 Hr.ISO
2 Hr.ISO
3 Hr.ISO
Treatment

The Effect of Perfusion Time on Total Histone Acetylation and Protein Yields in 2 Hour ISO Treated Animals

Since the 2 hour ISO treated animals appeared to show an increase in acetylation, 2 hour ISO treated animals and saline treated controls were used to determine if ISO treatment had an influence on the time course of histone acetylation. The hearts were perfused for 5, 10, 20, 40, and 80 minutes. Table 2 represents the acetylation as $CPM.mg^{-1}$ protein and the protein yields as $ug.g^{-1}$ tissue wet weight.

TABLE 2

Effect of Perfusion Time on on Histone Acetylation and Protein Yields in 2 Hour ISO Treated Animals.

	$CPM.mg^{-1}$ Protein		Yield $ug.g^{-1}$ Tissue Wet Weight	
Perfusion Time (Min.)	Saline Control	2 Hour ISO	Saline Control	2 Hour ISO
5	11,801 ± 432	10,931 ± 22	320.90 ± 12.11	229.54 ± 11.26 *
10	11,995 ± 1,779	7,780 ± 479	320.92 ± 24.99	255.77 ± 9.42 *
20	13,875 ± 143	12,910 ± 798	279.76 ± 5.95	252.10 ± 15.84
40	7,333 ± 866	6,725 ± 802	320.26 ± 12.16	295.33 ± 42.80
80	7,069 ± 196	7,597 ± 711	295.33 ± 42.80	283.24 ± 9.67

Values are means ± S.E.M.

* Indicates statistical significance using T-test ($p<.05$)

The results indicate a significant difference in incorporation at 10 minutes perfusion with the ISO treated group lower than the saline controls ($p<.05$). There was also a difference in protein yields between the saline and ISO animals after 5 and 10 minutes of perfusion with the ISO treated animals showing a significant decrease ($p<.05$).

The Effect of Perfusion Time on Total Acetylation in Histone Fractions in 2 Hour ISO Treated Animals

Figure 3 represents total radioactivity at each condition as determined by the sum of the four electrophoretically purified histone fractions. The only difference in radioactivity between the saline and ISO treated animals occurred after 80 minutes of perfusion. There was a significant decrease in the values for the ISO treated group ($p<.05$).

The Effect of Perfusion Time on Acetylation of Histone Fractions in 2 Hour ISO Treated Animals

The isolated nucleohistones were fractionated electrophoretically to examine alterations in specific histones. Figure 4 illustrates the radioactivity present ($CPMx10^3.mg^{-1}$ protein) in each of the four identified fractions, at the various perfusion times for 2 hour saline and ISO treated animals. The only difference in radioactivity between the saline and ISO treated animals was a significant decrease in fraction H2 after 80 minutes perfusion in the ISO treated group ($p<.05$). There was a general increase in incorporation in all fractions to 20 minutes of perfusion, followed by variable decreases.

FIGURE 3: The Effect of Perfusion Time on Total Radioactivity in Electrophoretically Purified Histones, determined by the sum of the four fractions at each perfusion time. The only significant difference was after 80 minutes of perfusion. Each point represents the mean of 3 experiments.

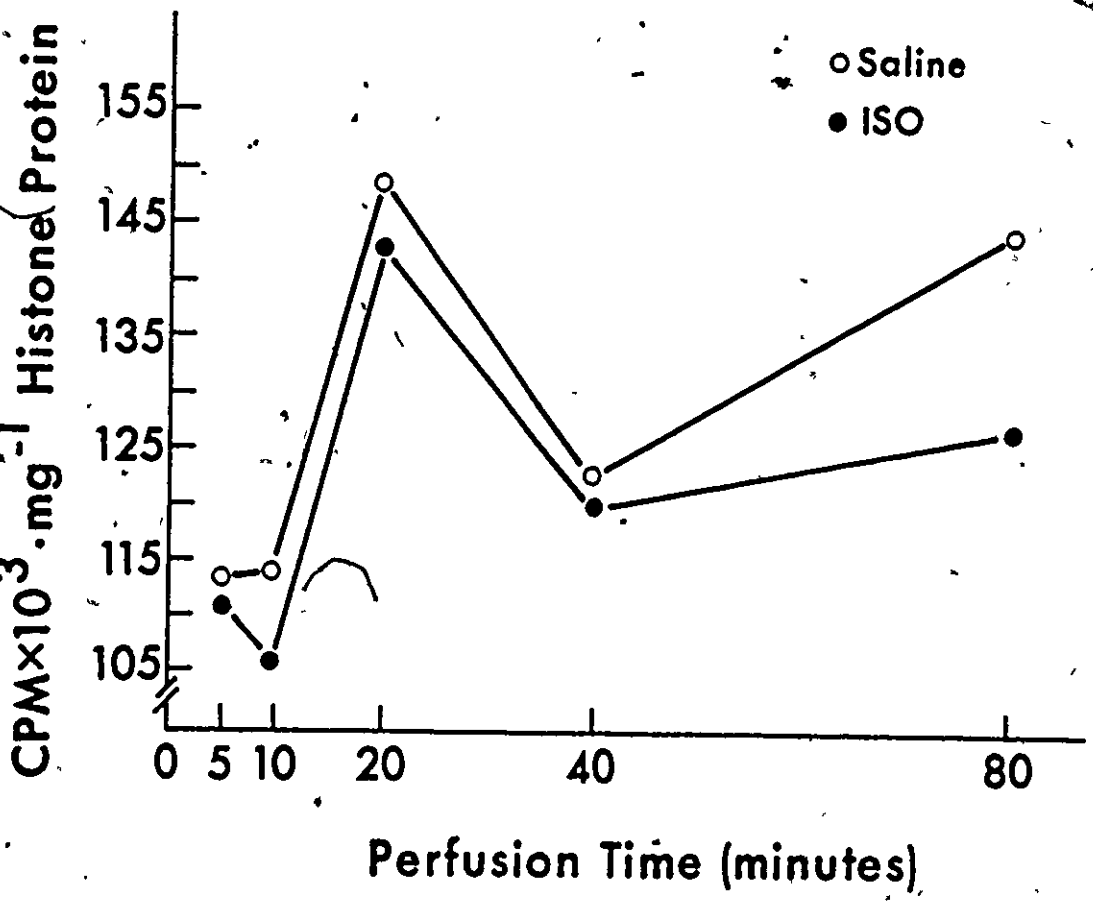
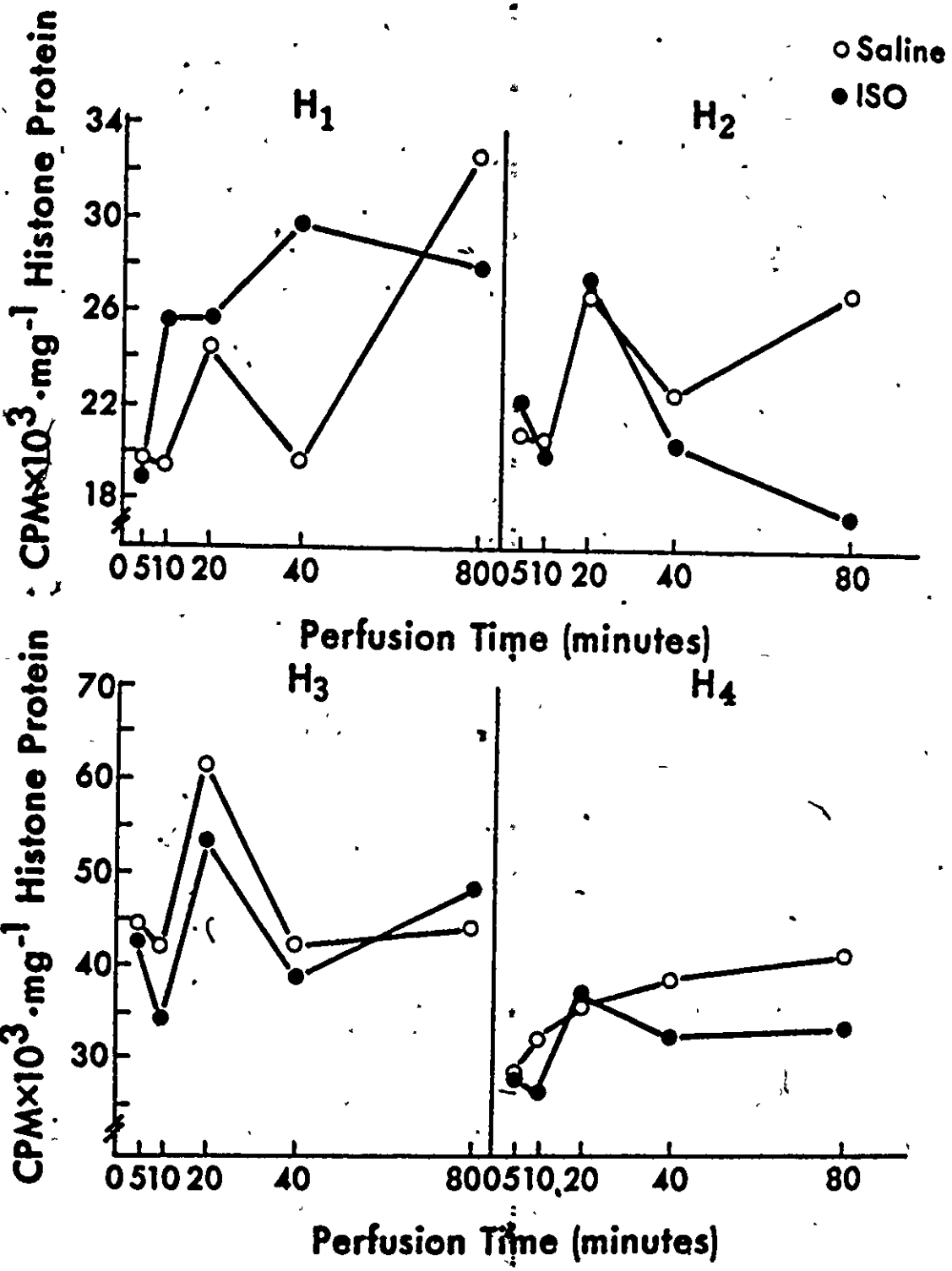

Saline
ISO
155
145
135
125
115
105
CPM×10^{3}.mg^{-1} Histone Protein
0
5
10
20
40
80
Perfusion Time (minutes)

FIGURE 4: The Effect of Perfusion Time on Acetylation in Purified Histone Fractions. [^{3}H] acetate labelled histones were fractionated electrophoretically and the radioactivity and protein in each fraction was quantified as described in the Method section. The results are expressed as CPMx10^3.mg^{-1} protein. The only significant difference was in fraction H2 after 80 minutes perfusion ($p < .05$) as determined by an independent student T-test. Each point represents the mean of 3 experiments.


Saline
ISO
H1
H2
H3
H4
CPM×10³.mg⁻¹ Histone Protein
34
30
26
22
18
70
60
50
40
30
0 5 10 20 40 80 0 5 10 20 40 80
Perfusion Time (minutes)

Fractions H3 and H4 were preferentially acetylated over fractions H1 and H2.

The Effect of ISO Concentration on Histone Acetylation

ISO was administered at 2 dose levels; 5 and 25mg.kg^{-1} body weight. All hearts were perfused for 20 minutes, 2 hours post injection. The results are shown in Figure 5. There were no significant differences in any of the fractions between the two dose levels of ISO. Fraction H3 was significantly lower in the animals treated with 25mg.kg^{-1} body weight when compared to saline treated animals ($p<.05$). Under all treatment conditions, fractions H3 and H4 were more acetylated than fractions H1 and H2. The pattern of incorporation was the same for both dose levels of ISO.

The Effect of Propranolol Treatment on Histone Acetylation

To determine if blocking the beta receptors of the heart had an effect on histone acetylation, animals were given subcutaneous injections of propranolol (25mg.kg^{-1} body weight). Figure 6 shows the values for propranolol treatment compared to saline controls. There were no significant differences between the saline and propranolol treated animals in any of the isolated fractions. The pattern of acetylation was unchanged after propranolol treatment. Once again, fractions H3 and H4 were more highly acetylated than fractions H1 and H2.

FIGURE 5: The Effect of 2 Hour Treatments of Saline, ISO (5mg.kg^{-1} body weight), and ISO (25mg.kg^{-1} body weight), on Histone Acetylation. There were no significant differences between the two levels of ISO treatment. Fraction H3 was significantly reduced in the ISO (25mg.kg^{-1} body weight) animals compared to the saline controls ($p<.05$). Each point represents the mean of 3 experiments.

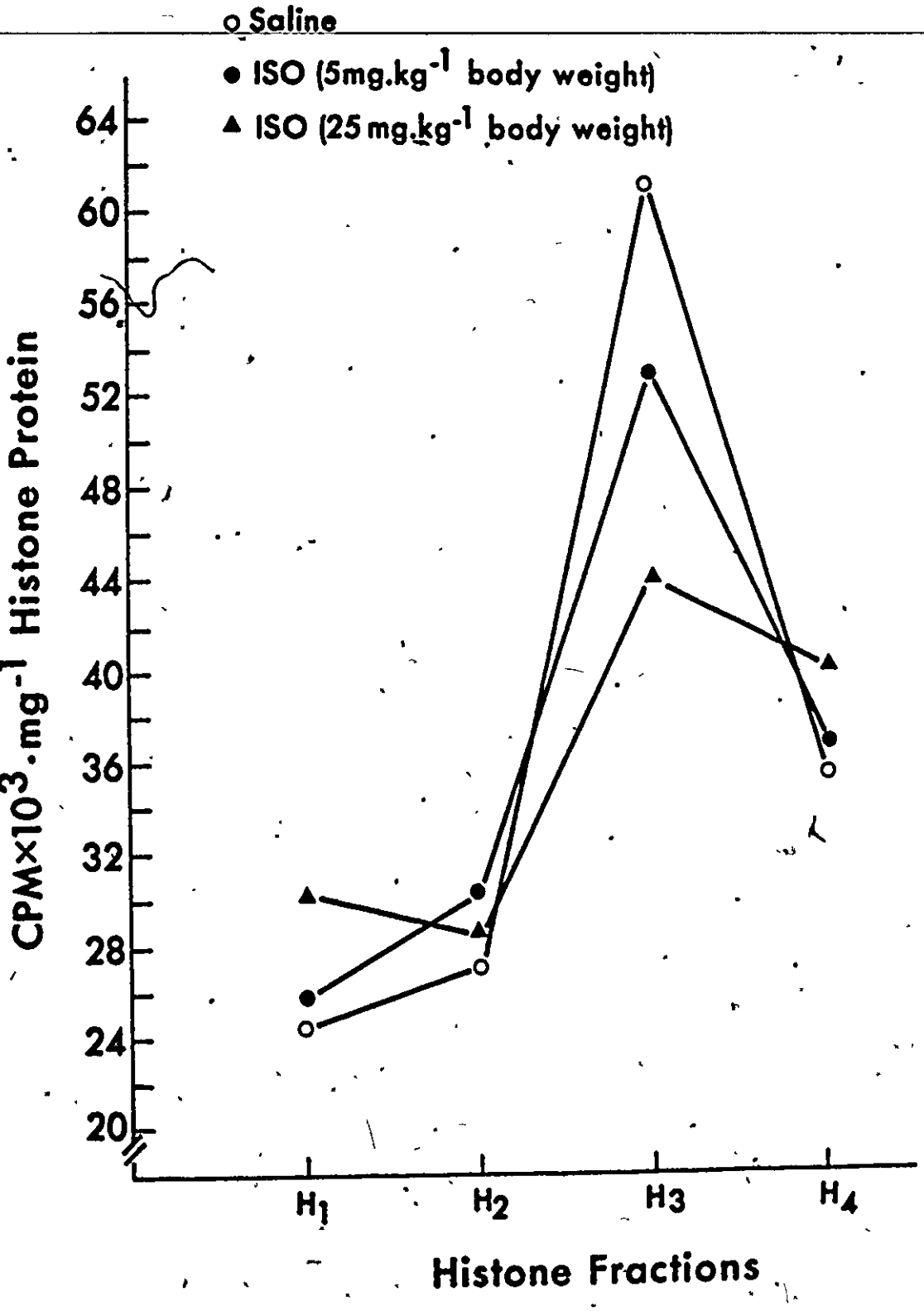
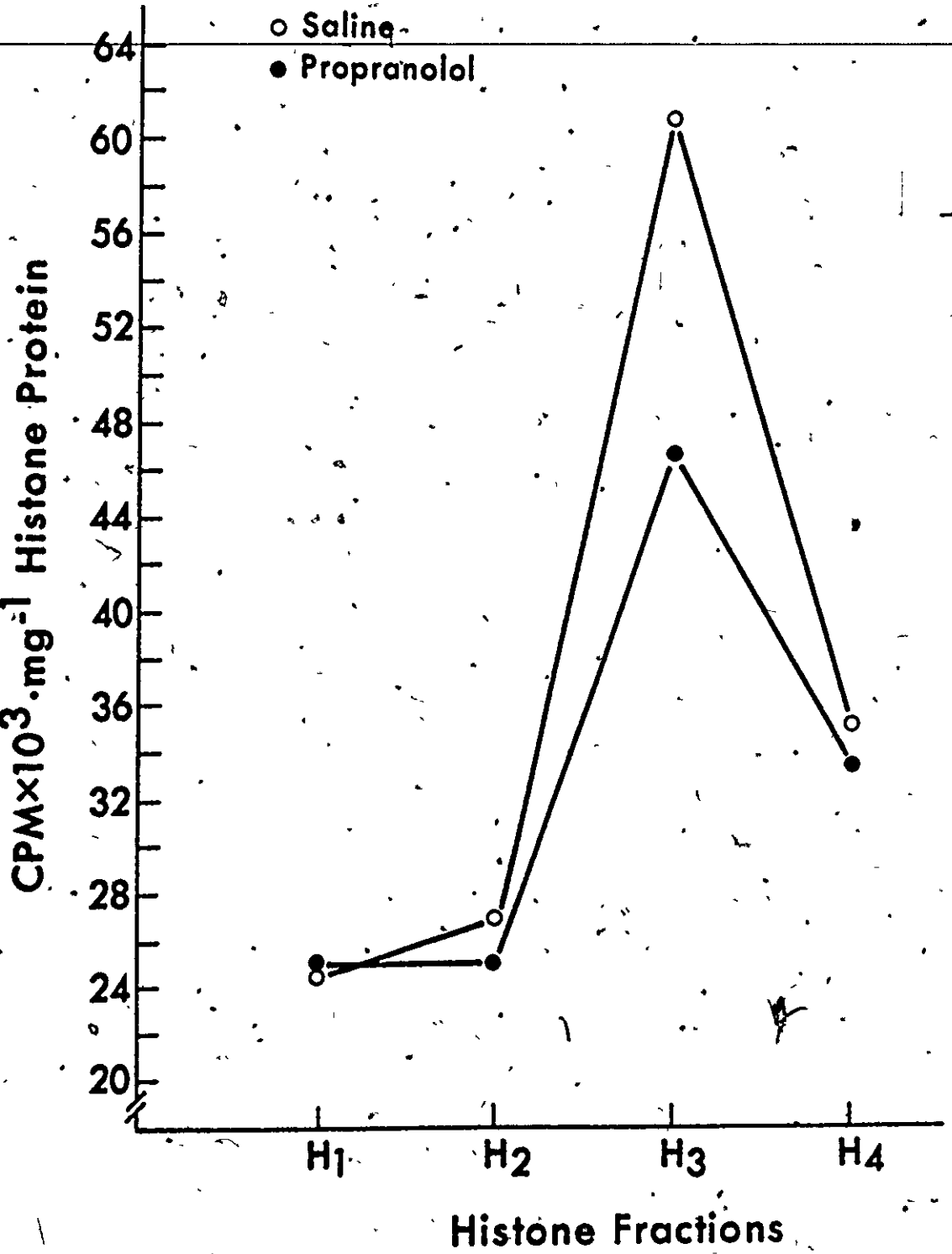

o Saline
● ISO (5mg.kg^{-1} body weight)
▲ ISO (25 mg.kg^{-1} body weight)
CPM×10^3.mg^{-1} Histone Protein
64
60
56
52
48
44
40
36
32
28
24
20
H1
H2
H3
H4
Histone Fractions

FIGURE 6: The Effect of 2 Hour Treatments of Saline, and Propranolol (25mg.kg^{-1} body weight) on Histone Acetylation. There were no significant differences between the saline and propranolol treated animals. Each point represents the mean of 3 experiments.


Saline
Propranolol
64
60
56
52
48
44
40
36
32
28
24
20
CPM×10³.mg⁻¹ Histone Protein
H1
H2
H3
H4
Histone Fractions

CHAPTER IV

DISCUSSION

The DNA of diploid eukaryotic cells is primarily located in the cell nucleus where it is ionically bound with basic proteins called histones, and acidic, non-histone chromosomal proteins. Histones are relatively small proteins with molecular weights ranging from 10,000 to 20,000 daltons (Delange et al., 1975). Five histone fractions have been identified on the basis of their amino acid compositions (H1, H2A, H2B, H3, and H4). Fractions H2A and H2B are slightly lysine rich and fractions H3 and H4 are arginine rich (Delange, et al., 1975). Histone proteins occur in near equimolar concentrations to DNA in eukaryotic nuclei (DePamphillis, et al., 1980). About 140 base pairs of DNA are complexed with two molecules each of histone H2A, H2B, H3 and H4 forming a core particle (Smulson, 1979; Champoux, 1978; Kornberg, 1977; McGhee, et al., 1976). The DNA is wrapped around the outside of the core of histones resulting in a supercoiled and compacted subunit. It is believed that the core particle is approximately 110 Å in diameter (Kornberg, 1977). The DNA segment between core particles is referred to as the linker region. The length of the linker is variable and is believed to be associated with one molecule of H1 (Champoux, 1978; Kornberg, 1977). The core particle plus the linker comprises a nucleosome

structure. The repetitive organization of nucleosomes is thought to be involved in maintaining the more stable structure of interphase chromatin and reduced DNA template activity.

Delange et al. (1975) have demonstrated that a remarkable conservation of amino acid sequences has been maintained throughout evolution. Apparently, there is little variation in amino acid composition of histones between organs of a given animal or between different species. It has been found that only 2% of the amino acid residues differ between pea seedlings and calf thymus histone H4. It has been suggested that this remarkable conservation of amino acid sequence indicates a similar role of histones in all organisms (Allfrey, 1968). The close association of histones with DNA suggests they may be implicated in functions involving the structural organization of chromatin and possibly as gene repressors or regulators.

Several lines of evidence suggest that chemical modifications of the nuclear proteins are involved in the activation of DNA template activity. In support of the notion that histones may have some degree of regulation, these nuclear proteins undergo acetylation of the N-terminal amino acid group and the ε- amino group of certain lysine residues, methylation of the ε- amino group of some lysine residues, and phosphorylation of specific serine residues

(Bradbury, 1975). Liew et al. (1972, 1973) have reported data suggesting that an increased acetylation and phosphorylation of chromosomal proteins occurred prior to an increase RNA polymerase activity and RNA synthesis.

Cousens et al. (1979) have shown that sodium butyrate inhibits the enzyme(s) responsible for histone deacetylation. Simpson et al. (1978) have used Dnase 1, which preferentially cleaves transcriptionally active DNA, in sodium butyrate incubated cells, and demonstrated an increased rate of DNA cleavage suggesting that there was an increase in transcription when deactylation was inhibited. These results support the view that histone acetylation induces an alteration of chromatin structure possibly leading to the promotion of transcription.

Isoproterenol is a synthetic adrenergic compound which activates beta receptors in the heart. The presence of the synthetic catecholamine at the cardiac sarcolemma causes the activiation of adenyl cyclase which converts ATP to cyclic AMP (Guyton, 1976). Cyclic AMP acts as a secondary messenger within the cell and translates the initial message of the hormone to the cellular components. The immediate effects of ISO are arterial hypotension and persistent tachycardia (Rona, et al., 1963). Several investigators have shown that consecutive low doses of ISO produces cardiac hypertrophy (Stanton, et al., 1967; Stanton, et al., 1969; Taylor, et al., 1977; Wood, et al., 1971).

In this investigation, ISO was used as a stimulus to initiate protein synthesis and compensatory hypertropy, and histone acetylation was examined in the early responses to hypertrophy.

Propranolol is a beta receptor blocker (Niarchos, et al., 1978). It blocks adrenergic activity by occupying the beta receptor sites. Propranolol was used in this study to determine the effects of beta blockade on histone acetylation as opposed to beta stimulation induced by ISO.

Animals were treated for 1, 2, and 3 hours with ISO (5mg.kg^{-1} body weight) and histone acetylation determined (Table 1). These results indicate no significant differences between the saline and ISO treated animals in either the radioactivity or protein yields obtained. The lack of a difference in the protein yields suggests that the isolation technique was consistent and reliable and that there was uniform sampling of the total population of histones.

Figures 1 and 2 show the data for the electrophoretically purified histone fractions following 1, 2, and 3 hours of ISO treatment. The isolated histones were electrophoretically purified into the component fractions to examine the response of the individual fractions. The results indicated a general increase 1 and 2 hours after ISO administration followed by a reduction after 3 hours, however, the differences were not statistically significant. Since other

investigators have found a 300-400% increase in RNA synthesis 3 hours after a single injection of ISO of a comparable dose, the increase in acetylation seen after 1 and 2 hours of ISO treatment are consistent with the suggestion that the covalent modification of histones preceeds an increase in transcriptional activity. The small sample coupled with large variability in the data, may be responsible for the lack of statistically significant differences, however, the trend observed may have been of biological importance. Fractions H3 and H4 were more highly acetylated than fractions H1 and H2 which is consistent with the findings of others (Liew, et al., 1973; Taylor, et al., 1976).

Due to the apparent increase in acetylation in the 2 hour ISO treated animals, this time was selected to examine the time course of histone acetylation and deacetylation. The yields of unpurified histones and incorporation present in these samples are seen in Table 2. The only significant difference between the saline and 2 hour ISO treated animals in the incorporation of the [3H] acetate was after 10 minutes perfusion with the ISO group being lower than the saline controls. The protein yields showed two significant differences between the controls and the ISO treated groups. These occurred at 5 and 10 minutes of perfusion and in both cases, the ISO was significantly reduced. The relatively consistent protein yields suggest that the isolation tech-

nique was reliable and the extended perfusion time did not affect the recovery of the nucleoproteins. Although no physical parameters of the hearts' performance were measured, there did not appear to be any noticeable differences in function between the saline and the ISO treated hearts. At the longest perfusion time (80 minutes) there was a tendancy for the hearts to decrease in contraction rate. This factor may contribute to the greater variability in the data at this perfusion time.

To determine the possibility that there may be a specific response to the ISO treatment, the histones were electrophoretically fractionated. The sum of the radioactivity present in the fractions in the saline and 2 hour ISO treated animals is shown in Figure 3. The only significant difference was found at 80 minutes of perfusion. The pattern of acetylation was similar for both groups. There was a rapid increase in incorporation between 10 and 20 minutes of perfusion which is consistent with data previously reported (Taylor, et al., 1976) followed by a decrease at 40 minutes. The data for the individual fractions is illustrated in Figure 4. There was a significant difference between the saline and 2 hour ISO treated animals in fraction H2 at 80 minutes of perfusion with the ISO treated tissue being significantly reduced compared to the control values. This difference may be a function of the perfusion time rather than a true biological phenomena.

A multitude of reasons can explain the apparent lack of stimulation produced by ISO treatment. One limiting factor of this investigation was the small sample size. The nature of the experiments with reference to the amount of tissue required for analysis, limited the sample size to 3 in each condition. It is possible that considering the variability involved in the methods, this sample size was too small to enable the detection of differences. The possibility exists that the ISO used may not have been physiologically active. However, this seems unlikely since previous studies from this laboratory and current experiments indicate that this batch of ISO is biologically active. If one assumes that the ISO used was active, it is possible that the increase in protein synthesis that follows ISO treatment is accomplished by increased translation of the mRNA matrices already present and that an increase in transcription of RNA species was not stimulated (Meerson, 1969). During temporary increases in the functional activity of an organ, it is likely that the process of translation is activated on pre-existing mRNA matrices, and that only during long periods of intense hyperfuction would there be an increase in transcription (Meerson, 1969). If this is true, one would not anticipate the stimulation of histone acetylation. Another possible explanation for the apparent lack of change is that covalent modification of nucleohistones, may be dose dependent. To

examine this possibility, a subsequent set of experiments were performed using a dose of 25mg.kg^{-1} body weight, representing a 5-fold increase.

The data comparing the 2 dosages of ISO administered and the saline controls is illustrated in Figure 5. There are no significant differences between the two dose levels of ISO and histone acetylation. These results suggest that insufficient dosage does not account for the apparent lack of stimulation. However, it may support the inactivity of the isoproterenol used in the study. The only significant difference in radioactivity between the ISO (25mg.kg^{-1} body weight) and the saline treated groups was in fraction H3 which was significantly reduced in the ISO treated animals.

In the above experiments, ISO (a beta receptor stimulator) was used in an attempt to examine subsequent changes in histone actylation. Since the experimental results were not as expected, a subsequent experiment was performed using a drug which supresses beta receptor activity in the heart. The most important effects of beta-blockade are on the cardiovascular system. Propranolol can effectively block the effects of the sympathetics on the heart. Administration of a single injection of propranolol produces a decrease in heart rate and prolongation of mechanical systole (Goodman, et al., 1970). Propranolol was administered and allowed to circulate for 2 hours.

These data are shown in Figure 6. There were no significant differences between the propranolol and saline treated animals suggesting that beta blockade did not affect histone acetylation. Fractions H3 and H4 were again more highly acetylated than fractions H1 and H2. This experiment may have been more useful if the propranolol treatment had been followed by administration of ISO or vice versa, as Goodman et al. (1970) reported that beta-receptor blockade has little effect on the normal heart at rest, but may have profound effects when sympathetic output is high.

These results do not support the work of Liew et al. (1972, 1973) who have reported increases in histone acetylation preceeding increases in RNA synthesis. It is believed by many researchers that the post-synthetic covalent modification of histone proteins is one of the earliest events in gene activiation. These data appear contrary to this hypothesis. The possibility exists that the reduced histone acetylation observed in these experiments may be a protective mechanism to stablize the DNA. Felsenfeld et al. (1975) have suggested that most chromatin proteins function to protect DNA from nuclease digestion. An increase in the covalent modification of nucleohistones results in weakening the DNA-histone interaction and thereby exposing segments of DNA to serve as a template for transcription (Taylor, et al., 1976). The exposed segments may then be more susceptable to enzymatic degradation. If this

is the case, decreasing the acetylation of nucleohistones may serve as a biological mechanism to protect the DNA.

Although the results of this investigation do not support the hypothesized role of histones as gene regulators, they may implicate the histones as serving a protective function. The data attained in this study are too inconclusive to confirm either of these previously hypothesized functions of histone proteins.

REFERENCES

1. Allfrey, V.G. "Some Obervations on Histone Acetylation and Its Temporal Relationship to Gene Activation." In Regulatory Mechanisms for Protein Synthesis in Mammalian Cells, New York, New York: Academic Press Inc., 1968, pp. 65-100.

2. Bradbury, E. M. "Histones in Chromosomal Structure and Control of Cell Division." In The Structure and Function of Chromatin, New York, New York: Associated Scientific Publishers, 1975, pp. 131-148.

3. Champoux, J. J. "Proteins that Affect DNA Conformation." Annual Review of Biochemistry, 1978, 47, 449-479.

4. Cousens, L. S., Gallwitz, D., and Alberts, B. M. "Different Accessibilities in Chromatin to Histone Acetylase." The Journal of Biological Chemistry, 1979, 254(5), 1716-1723.

5. Cutilletta, A. F., Dowell, R. T., Rudnik, M., Arcilla, R. A., and Zak, R. "Regression of Myocardial Hypertrophy I. Experimental Model; Changes in Heart Weight, Nucleic Acids, and Collagen." Journal of Molecular and Cellular Cardiology, 1975, 7, 767-781.

6. Delange, R. J., and Smith, E. L. "Histone Function and Evolution as Viewed by Sequence Studies." In The Structure and Function of Chromatin, New York, New York: Associated Scientific Publishers, 1975, pp. 59-69.

7. DePamphilis, M. L., and Wassarman, P. M. "Replication of Eukaryotic Chromosomes: A Close-up of the Replication Fork." Annual Review of Biochemistry, 1980, 49, 627-666.

8. Fanburg, B. L., and Posner, B. I. "Ribonucleic Acid Synthesis in Experimental Cardiac Hypertrophy in Rats." Circulation Research, 1968, 23, 123-135.

9. Fanburg, B. L. "Experimental Cardiac Hypertrophy." The New England Journal of Medicine, 1970, 282 (13), 723-731.

10. Felsenfeld, G., Axel, R., Cedar, H., and Sellner-Webb, B. "The Specific Template Activity of Chromatin." In The Structure and Function of Chromatin, New York, New York: Associated Scientific Publishers, 1975, pp. 29-40.

11. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., and Chambon, P. "Folding of the DNA Double Helix in Chromatin - Like Structures from Simian Virus 40." Proceedings of the National Academy of Science USA, 1975, 72(5), 1843-1847.

12. Goodman, L. S., and Gilman, A. In The Pharmacological Basis of Therapeutics, 4th Edition, New York, New York: Macmillan Co., 1970, pp. 565-570.

13. Guyton, A. C. In Textbook of Medical Physiology, 5th Edition, Philadelphia, Pensylvania: W.B. Saunders Co., 1976, pp. 990.

14. Kallfelt, B. J., Hjalmarson, A. C., and Isaksson, O. G. "In Vitro Effects of Catecholamines on Protein Synthesis in Perfused Rat Heart." Journal of Molecular and Cellular Cardiology, 1976, 8, 787-802.

15. Koide, T., and Rabinowitz, M. "Biochemical Correlate of Cardiac Hypertrophy II. Increased Rate of RNA Synthesis in Experimental Cardiac Hypertrophy in the Rate." Circulation Research, 1969, 24, 9-18.

16. Kornberg, R. D. "Structure of Chromatin." Annual Review of Biochemistry, 1977, 46, 931-954.

17. Krebs, H. A., and Henseleit, K. Z. Hoppo-Seyle Z., 1932, 120, 33.

18. Liew, C. C., Liu, D. K., and Gornall, A. G. "Effects of Aldosterone on RNA Polymerase in Rat Heart and Kidney Nuclei." Endocrinology, 1972, 90, 488-495.

19. Liew, C. C., Suria, D., and Gornall, A. G. "Effects of Aldosterone on Acetylation and Phosphorylation of Chromosomal Proteins." Endocrinology, 1973, 93, 1025-1034.

20. Lilley, D. M. J., Howarth, O. W., and Clark, V. M. "The Existence of Random Coil N-Terminal Peptides - 'Tails' - in Native Histone Complexes." FEBS Letters, 1976, 62(1), 7-10.

21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. "Protein Measurements with the Folin Phenol Reagent." Journal of Biological Chemistry, 1951, 193, 265-275.

22. McGhee, J. D., and Felsenfeld, G. "Nucleosome Structure." Annual Review of Biochemistry, 1980, 49, 1115-1156.

23. Meerson. "Replication, Transcription, and Translation in the Myocardium in Compensatory Hypertension and Heart Failure." Supplement II to Circulation Research, 1967, 24 and 25, 100-114.

24. Morgan, H. E., Henderson, M. J., Regen, D. M., and Park, C. R. "Regulation of Glucose Uptake in Muscle I. The Effects of Insulin and Anoxia on Glucose Transport and Phosphorylation in the Isolated, Perfused Heart of Normal Rats." The Journal of Biological Chemistry, 1961, 236(2), 253-261.

25. Morkin, E., and Ashford, T, P. "Myocardial DNA Synthesis in Experimental Cardiac Hypertrophy." American Journal of Physiology, 1968, 215(6), 1409-1413.

26. Morkin, E. "Activation of Synthetic Processes in Cardiac Hypertrophy." Supplement II to Circulation Research, 1974, 34 and 35, 37-47.

27. Nair, K. G., Cutilletta, A. F., Zak, R., Koide, T., and Rabinowitz, M. "Biochemical Correlates of Cardiac Hypertrophy I. Experimental Model; Changes in Heart Weight, RNA Content, and Nuclear RNA Polymerase Activity." Circulation Research, 1968, 23, 451-462.

28. Niarchos, A. P., Tahmooressi, P., and Tarazi, R. C. "Comparision of Heart Rate and Blood Pressure in Response to Amyl Nitrate, Isoproterenol, and Standing Before and During Acute Beta-Adrenergic Blockade with Intravenous Propranolol." American Heart Journal, 1978, 96, 47-53.

29. Panyim, S., and Chalkley, R. "High Resolution Acrylamide Gel Electrophoresis of Histones." Archives of Biochemistry and Biophysics, 1969, 130, 337-346.

30. Pogo, B. G. T, Allfrey, V. G., and Mirsky, A. E. "RNA Synthesis and Histone Acelytation during the Course of Gene Activation in Symphocytes." National Academy Washington Proceedings, 1966, 55, 805-812.

31. Pogo, B. G. T., Pogo, A. O., Allfrey, V. G., and Mirsky, A. E. "Changing Patterns of Histone Acetylation and RNA Synthesis in Regeneration of the Liver." National Academy of Science Proceedings, 1968, 59, 1337-1348.

32. Rabinowitz, M., Nair, K. G., and Zak, R. "Cellular and Subcellular Basis of Cardiac Hypertrophy." Medical Clinics of North America, 1970, 54(1), 211-220.

33. Ris, H. "Chromosomal Structure as seen by Electron Microscopy." In The Structure and Function of Chromatin, New York, New York: Associated Scientific Publishers, 1975, pp 7-23.

34. Rona, G., Chappel, C. I., Balazs, T., and Gaudry, R. "The Effect of Breed, Age, and Sex on Myocardial Necrosis Produced by Isoproternol in the Rat." Journal of Gerontology, 1959, 14, 169-173.

35. Rona, G., Kahn, D. S., and Chappel, C. I. "Studies on Infarct - Like Myocardial Necrosis Produced by Isoproterenol: A Review." Revue Canadienne de Biologie, 1963, 22, 241-255.

36. Schreiber, S. D., Oratz, M., and Rothschild, M. A. "Effect of Acute Overload on Protein Synthesis in Cardiac Muscle Microsomes." American Journal of Physiology, 1967, 213(6), 1552-1555.

37. Simpson, R. T. "Structure of Chromatin Containing Extensively Acetylated H3 and H4." Cell, 1978, 13 691-699.

38. Smulson, M. "Nuclear Protein Modification and Nucleosome Chromatin Structure and Function." Trends in Biochemical Science, 1979, 4(4), 225-228.

39. Stanton, H. C., and Schwartz, A. "Effects of a Hydrazine Monoamine Oxidase Inhibitor (Phenelzine) on Isoproterenol - Induced Myocardiopathies in the Rat." The Journal of Pharmacology and Experimental Therapeutics, 1967, 157(3), 649-658.

40. Stanton, H. C., Brenner, G., and Mayfield, E. D. "Studies on Isoproterenol - Induced Cardiomegaly in Rats." American Heart Journal, 1969, 77, 72-80.

41. Stein, G., Stein, J. S., and Kleinsmith, L. J. "Chromosomal Proteins and Gene Regulation." Scientific American, 1975, 232, 46-57.

42. Takenaka, F., and Higuchi, M. "High-Energy Phosphate Contents of Subepicardium and Subendocardium in the Rat Treated with Isoproterenol and Some Other Drugs." Journal of Molecular and Cellular Cardiology, 1974, 6, 123-135.

43. Taylor, P. B., and Liew, C. C. "Acetylation of Nuclear Proteins in the Isolated Perfused Rat Heart." Basic Research in Cardiology, 1976, 71, 27-35.

44. Taylor, P. B., Swanson, C. T., and Draisey, T. F. "Quantitative Histological Changes in Isoproterenol Induced Cardiac Growth." Research Communications in Chemical Pathology and Pharmacology, 1977, 17(2), 341-344.

45. Wangh, L., Ruiz-Carrillo, A., and Allfrey, V. G. "Separation and Analysis of Histone Subfractions Differing in their Degree of Acetylation: Some Correlations with Genetic Activity in Development." Archives of Biochemistry and Biophysics, 1972, 150, 44-56.

46. Wikman-Coffelt, T., Parmley, W. W., and Mason, D. T. "The Cardiac Hypertropy Process. Analysis of Factors Determining Pathological vs Physiological Development." Circulation Research, 1979, 45(6), 697-707.

47. Wood, W. G., Lindenmayer, G. E., and Schwartz, A. "Myocardial Synthesis of Ribonucleic Acid I. Stimulation by Isoproterenol." Journal of Molecular and Cellular Cardiology, 1971, 3, 127-138.

APPENDIX A

Perfusion Buffer

1. Krebs, Henseleit Bicarbonate Buffer (24)

Salt Stocks	%(gm/100ml)	Molar Conc.	ml Used (x3)
NaCl	.9	.154	1000 (x3)
KCl	1.15	.154	40 (x3)
$CaCl_2$	1.22	.110	36 (x3)
KH_2PO_4	2.11	.154	10 (x3)
$MgSO_4 .7H_2O$	3.82	.154	10 (x3)
$Na_2EDTA .2H_2O$	3.72	.100pH7.4	6 (x3)
$NaHCO_3$	1.30	.154	6 (x3)
Substrate (Glucose)	.391		

2. Procedure for Buffer Preparation

Add 27gm NaCl) - Dilute to 3 litres with
11.8gm Glucose) double distilled water.

Add Appropriate volumes of stock salt sol'n (KCl, $CaCl_2$, KH_2PO_4, EDTA)

Gas with 95% O_2 & 5% CO_2 for 5 - 10 minutes.

Add Appropriate vol. of $NaHCO_3$ slowly while buffer is being oxygenated.

APPENDIX B

ISOLATION OF HISTONE PROTEINS

1. Solutions:

Sol'n A - 10mM TRIS-HCl (pH 7.2)
- 1mM $MgCl_2$
- 0.25M Sucrose
- 0.1M phenylmethylsulfonyl-flouride (PMSF)

Sol'n B - 10mM TRIS-HCl (pH 7.2)
- 1mM $MgCl_2$
- 0.25M Sucrose
- 0.1M PMSF
- 0.1% Triton X-100

Sol'n C - 10mM TRIS-HCl (pH 7.2)
- 1mM $MgCl_2$
- 2.2M Sucrose
- 0.1M PMSF

Sol'n D - 10mM TRIS-HCl (pH 7.2)
- 0.14 M NaCl
- 0.1M PMSF

Sol'n E - 0.25 N HCl
- 0.1M PMSF

2. Procedure:

Pulse label hearts

Trim atria, fat, and connective tissue and weigh

Mince and homogenize hearts in 2 volumes of Sol'n A, spin 3000 rpm 10 minutes

Homogenize pellet in Sol'n A, filter through 2 layers of fine nylon, spin 3000 rpm 10 minutes

Suspend Pellet in Sol'n B, spin 3000 rpm 10 minutes

Suspend pellet in Sol'n C, underlay with 5ml Sol'N C, spin 25,000 rpm 60 minutes

Remove supernatent, rinse with Sol'n A, wipe dry

Suspend pellet in Sol'n A, spin 3000 rpm 10 minutes

(2x) Suspend pellet in Sol'n D, sit on ice 10 minutes, spin 10,000 rpm 10 minutes

(2x) Extract pellet in 1.5ml Sol'n E 30 minutes, spin 14,000 10 minutes

Precipitate overnight at 4°C in 10 volumes of ice cold acetone.

Recover precipitate by centrifugation, dry, solubilize in 300ul of 0.01 N HCl in 15% sucrose.

APPENDIX C

PROTEIN QUANTIFICATION

(Lowry, et al., 1951)

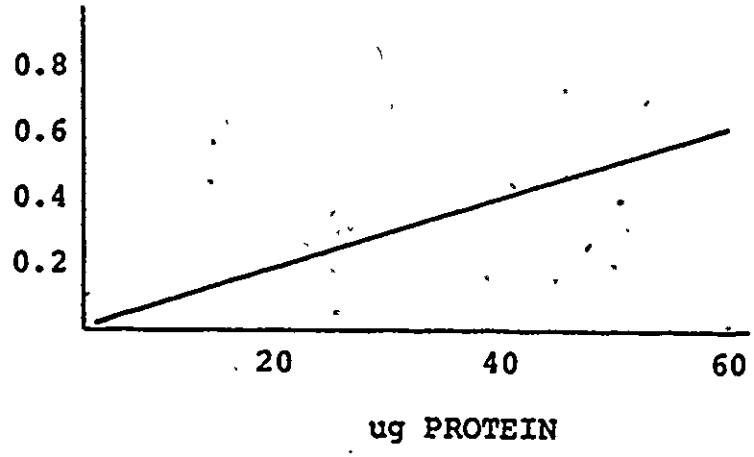
1. Chemicals:
   a) 2% Na_2CO_3 in 0.1 N NaOH
   b) 1% $CuSO_4.5H_2O$
   c) 2% Na-K Tartrate (cold)
   d) Phenol folin reagent

2. Standard: Calf thymus histone 1mg/~~ml~~ sol'n

3. Sol'n C: Prepare fresh daily in this order
   a) 0.5ml 1% $CuSO_4$
   b) 0.5ml 2% Na-K Tartrate
   c) 50ml 2% Na_2CO_3 in 0.1 N NaOH

4. Assay:
   a) make sample volume up to 0.4ml with 0.1 N NaOH
   b) add 2ml Sol'n C, wait 10 minutes at room temperature
   c) add 0.2ml Phenol folin reagent, agitate, and wait 30 minutes at room temperature.
   d) read O.D. at 750nm

5. Standard Curve:



APPENDIX D

ELECTROPHORESIS STOCK AND PROCEDURES

(Panyim, et al., 1969)

1. Solutions:

Sol'n A - 0.4% bisacrylamide
- 60% acrylamide

Sol'n B - 43.2% glacial acetic acid
- 4% TEMED

Sol'n C - 10 M urea

Running Buffer - 0.9 N acetic acid

Stain - 0.1% Amido Black
- 7% Acetic acid
- 20% ethanol

Destaining Sol'n - 7% acetic acid

2. Preparation of Gel Solution:

a) add 2 parts Sol'n A, 1 part Sol'n B, 5 parts Sol'n C to an erlenmeyer flask
b) cap with parafilm and deairate
c) add ammonium persulfate crystals and dissolve by gentle swirling

3. Casting of Gels:

a) mark gel tubes at 9.5cm
b) add gel sol'n with pasteur pipette
c) add 200ul of H_2O to flatten gel
d) polymerize overnight at room temperature

4. Pre-electrophoresis:

a) place gel tubes in electrophoresis apparatus
b) fill upper and lower chambers with running buffer
c) add 5ul of tracking dye to the top of one of the gels
d) set current at 1.75mA.gel^{-1} and run til tracking dye is eluted

5. Electrophoresis of Protein Sample:
   a) carefully layer a 60ug protein sample on the top of the gel using a Hamilton syringe
   b) add 5ul of tracking dye to the top of one of the gels
   c) set current at 1.75mA.gel-1 and run til 1 hour after tracking dye is eluted

6. Staining Gels:
   a) remove gels from tubes and place in test tubes
   b) fill test tubes with staining sol'n and stain overnight.

7. Destaining Gels:
   a) destain by diffusion in 7% acetic acid

APPENDIX E

DETERMINATION OF PROTEIN IN HISTONE FRACTIONS

(Wangh, et al., 1972)

1. Chemicals: dimethyl sulfoxide

2. Procedure:
   a) slice and quarter bands in gel and add 0.6ml of dimethyl sulfoxide
   b) elute colour at 40°C for 72 hours
   c) read optical densities at 615nm

APPENDIX F

DETERMINATION OF RADIOACTIVITY IN HISTONE FRACTIONS

1. Chemicals: 30% hydrogen peroxide

2. Procedure:
   a) slice bands in gel and digest in 0.5ml of 30% H_2O_2 at 40°C overnight
   b) add 10ml of high performance cocktail, dark adapt for 72 hours, count

APPENDIX G

INDIVIDUAL DATA

Effect of ISO Treatment Time on Histone Acetylation (CPM.mg^{-1} PROTEIN)

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
5053	2913	2747	2634
3356	6834	8177	1405
7355	4478	5172	4529
$\bar{x}$ = 5255 ± 1158	$\bar{x}$ = 4742 ± 1139	$\bar{x}$ = 5365 ± 1570	$\bar{x}$ = 2856 ± 908

Effect of ISO Treatment Time on Histone Protein Yields (ug.g^{-1} TISSUE WET WEIGHT)

Saline	1 Hr. ISO	2 Hr. ISO	3Hr. ISO
123.48	111.41	102.77	91.15
89.95	112.15	123.21	102.16
108.20	111.69	121.63	120.25
$\bar{x}$ = 107.21 ± 9.69	$\bar{x}$ = 111.75 ± 0.22	$\bar{x}$ = 115.85 ± 6.57	$\bar{x}$ = 104.52 ± 8.48

* Mean values are ± S.E.M.

Effect of ISO Treatment Time on the Acetylation of Electrophoretically Purified Histones (CPMx10^3.mg^{-1} HISTONE PROTEIN)

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
5,764.2	9,037.5	11,840.0	23,649.2
24,188.7	36,955.0	39,393.0	16,787.5
21,867.5	18,445.0	19,857.5	18,050.0
$\bar{x}$ = 17,273.5 ± 5,793.5	$\bar{x}$ = 21,479.2 ± 8,200.6	$\bar{x}$ = 23,696.8 ± 8,182.2	$\bar{x}$ = 19,495.6 ± 2,108.6

* Mean values are ± S.E.M.

Effect of ISO Treatment Time on Acetylation in Histone Fractions (CPMx10^3.mg^{-1} HISTONE PROTEIN)

Fraction H1

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
1.476	0.547	5.261	17.514
18.496	41.525	37.201	17.328
12.982	9.611	10.843	11.770
$\bar{x}$ = 10.985 ± 5.014	$\bar{x}$ = 17.228 ± 12.427	$\bar{x}$ = 17.768 ± 9.849	$\bar{x}$ = 15.537 ± 1.884

Fraction H2

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
1.982	3.014	3.280	10.378
10.936	18.185	20.271	6.983
11.020	9.268	10.096	7.658
$\bar{x}$ = 7.979 ± 2.999	$\bar{x}$ = 10.156 ± 4.402	$\bar{x}$ = 11.216 ± 4.937	$\bar{x}$ = 8.340 ± 1.038

Fraction H3

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
12.248	18.858	18.967	39.032
37.443	52.418	57.950	26.759
37.285	33.571	37.197	31.149
$\bar{x}$ = 28.992 ± 8.372	$\bar{x}$ = 34.949 ± 9.712	$\bar{x}$ = 38.038 ± 11.261	$\bar{x}$ = 32.313 ± 3.590

Fraction H4

Saline	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
3.713	8.142	17.808	23.234
27.424	34.968	39.234	14.797
20.310	17.670	17.287	18.323
$\bar{x}$ = 17.149 ± 7.025	$\bar{x}$ = 20.254 ± 7.856	$\bar{x}$ = 24.776 ± 7.230	$\bar{x}$ = 18.785 ± 2.446

*Mean values are ± S.E.M.

Effect of Perfusion Time on Histone Acetylation
(CPM.mg^{-1} PROTEIN)

Perfusion Time (Minutes)	Saline	ISO Treated
5	12,476.19	10,928.21
	11,932.14	10,971.58
	10,996.77	10,893.45
	$\bar{x}$ = 11,801.70 ± 432.00	$\bar{x}$ = 10,931.10 ± 22.63
10	8,825.74	7,526.38
	12,053.61	7,105.74
	14,988.55	8,709.03
	$\bar{x}$ = 11,956.00 ± 1,779.74	$\bar{x}$ = 7,780.36 ± 479.95
20	14,158.94	13,847.82
	13,696.93	11,323.05
	13,770.69	13,560.58
	$\bar{x}$ = 13,875.50 ± 143.26	$\bar{x}$ = 12,910.50 ± 797.99
40	8,316.99	8,330.43
	5,606.59	5,933.44
	8,077.53	5,913.63
	$\bar{x}$ = 7,333.70 ± 866.31	$\bar{x}$ = 6,725.80 ± 802.32
80	7,330.38	6,279.14
	6,684.80	8,720.85
	7,192.16	7,793.90
	$\bar{x}$ = 7,069.13 ± 196.29	$\bar{x}$ = 7,597.96 ± 711.66

* Mean values are ± S.E.M.

Effect of Perfusion Time on Histone Protein Yields
(ug.g^{-1} TISSUE WET WEIGHT)

Perfusion Time (Minutes)	Saline	ISO Treated
5	325.03	240.13
	339.51	207.03
	298.17	241.47
	$\bar{x}$ = 320.90 ± 12.10	$\bar{x}$ = 229.53 ± 11.28
10	282.62	247.44
	312.26	245.30
	367.87	274.56
	$\bar{x}$ = 320.93 ± 24.99	$\bar{x}$ = 255.77 ± 9.44
20	268.34	269.12
	282.59	220.45
	288.35	266.73
	$\bar{x}$ = 279.77 ± 5.97	$\bar{x}$ = 252.10 ± 15.82
40	334.69	357.13
	296.09	315.74
	330.00	213.13
	$\bar{x}$ = 320.27 ± 12.16	$\bar{x}$ = 295.30 ± 42.80
80	290.83	264.69
	302.05	297.27
	240.14	287.75
	$\bar{x}$ = 277.67 ± 19.07	$\bar{x}$ = 283.27 ± 9.68

* Mean values are ± S.E.M.

Effect of Perfusion Time on the Acetylation of Electrophoretically Purified Histones
(CPMx10^3.mg^{-1} HISTONE PROTEIN)

Perfusion Time (Minutes)	Saline	ISO Treated
5	120.01	104.25
	113.78	113.26
	107.12	119.64
	$\bar{x}$ = 113.64 ± 3.72	$\bar{x}$ = 112.38 ± 4.47
10	87.79	94.82
	113.16	110.32
	141.45	114.22
	$\bar{x}$ = 114.13 ± 15.50	$\bar{x}$ = 106.45 ± 5.93
20	151.96	138.01
	149.34	136.54
	144.54	155.65
	$\bar{x}$ = 148.61 ± 2.18	$\bar{x}$ = 143.40 ± 6.14
40	114.84	122.42
	125.29	130.73
	129.04	109.23
	$\bar{x}$ = 123.06 ± 4.25	$\bar{x}$ = 120.79 ± 6.26
80	153.61	118.73
	140.61	130.33
	141.51	132.05
	$\bar{x}$ = 145.24 ± 4.19	$\bar{x}$ = 127.04 ± 4.18

* Mean values are ± S.E.M.

Effect of Perfusion Time on Acetylation in Histone Fractions ($CPM \times 10^3 \cdot mg^{-1}$ HISTONE PROTEIN)

Fraction H1

Perfusion Time (Minutes)	Saline	ISO Treated
5	15.20	18.70
	23.66	18.64
	20.27	19.55
	$\bar{x} = 19.71 \pm 2.46$	$\bar{x} = 18.96 \pm 0.29$
10	13.08	23.62
	23.13	26.28
	22.20	26.75
	$\bar{x} = 19.47 \pm 3.21$	$\bar{x} = 25.55$ 0.98
20	30.13	23.24
	22.92	32.83
	20.51	20.93
	$\bar{x} = 24.52 \pm 2.89$	$\bar{x} = 25.67 \pm 3.64$
40	18.77	19.93
	22.21	33.38
	18.11	34.13
	$\bar{x} = 19.70 \pm 1.27$	$\bar{x} = 29.15 \pm 4.61$
80	45.62	21.89
	26.18	32.55
	26.63	30.02
	$\bar{x} = 32.81 \pm 6.41$	$\bar{x} = 28.15 \pm 3.22$

Fraction H2

Perfusion Time (Minutes)	Saline	ISO Treated
5	19.85	20.30
	23.81	23.36
	18.92	22.81
	$\bar{x}$ = 20.86 ± 1.50	$\bar{x}$ = 22.16 ± 0.94
10	15.98	15.90
	16.94	23.22
	29.06	21.12
	$\bar{x}$ = 20.66 ± 4.21	$\bar{x}$ = 20.08 ± 2.18
20	24.05	20.60
	37.09	21.97
	20.23	40.37
	$\bar{x}$ = 27.12 ± 5.10	$\bar{x}$ = 27.65 ± 6.37
40	18.07	24.05
	24.00	24.47
	26.10	13.10
	$\bar{x}$ = 22.72 ± 2.40	$\bar{x}$ = 20.54 ± 3.72
80	30.72	19.36
	21.74	16.48
	28.91	16.59
	$\bar{x}$ = 27.12 ± 2.74	$\bar{x}$ = 17.48 ± 0.94

Fraction H3

Perfusion Time (Minutes)	Saline	ISO Treated
5	45.03	39.50
	47.63	43.62
	40.80	44.90
	$\bar{x}$ = 44.49 ± 2.00	$\bar{x}$ = 42.67 ± 1.63
10	33.61	34.39
	35.52	30.78
	57.15	37.74
	$\bar{x}$ = 42.09 ± 7.55	$\bar{x}$ = 34.30 ± 2.01
20	64.31	57.95
	56.03	42.77
	63.86	58.51
	$\bar{x}$ = 61.40 ± 2.69	$\bar{x}$ = 53.08 ± 5.16
40	41.44	42.32
	37.90	40.36
	47.23	33.06
	$\bar{x}$ = 42.19 ± 2.72	$\bar{x}$ = 38.58 ± 2.82
80	45.68	46.57
	46.71	44.56
	39.91	53.66
	$\bar{x}$ = 44.10 ± 2.12	$\bar{x}$ = 48.26 ± 2.76

Fraction H4

Perfusion Time (Minutes)	Saline	ISO Treatment
5	39.92	25.75
	18.67	27.64
	27.13	32.38
	$\bar{x}$ = 28.57 ± 6.18	$\bar{x}$ = 28.59 ± 1.97
10	25.13	20.91
	37.58	30.03
	33.05	28.61
	$\bar{x}$ = 31.92 ± 3.64	$\bar{x}$ = 26.52 ± 2.83
20	33.52	36.22
	33.31	38.97
	39.93	35.84
	$\bar{x}$ = 35.59 ± 2.17	$\bar{x}$ = 37.01 ± 0.99
40	36.56	36.12
	41.19	32.52
	37.61	28.94
	$\bar{x}$ = 38.45 ± 1.40	$\bar{x}$ = 32.53 ± 2.07
80	31.59	30.91
	45.98	36.73
	46.06	31.79
	$\bar{x}$ = 41.21 ± 4.81	$\bar{x}$ = 33.14 ± 1.81

* Mean values are ± S.E.M.

Effect of 2 Hour Treatments with Saline, ISO (5mg.kg^{-1} BODY WEIGHT), ISO (25mg.kg^{-1} BODY WEIGHT) and Propranolol (25mg.kg^{-1} BODY WEIGHT) on Acetylation in Histone Fractions (CPMx10^3.mg^{-1} HISTONE PROTEIN)

Fraction	Saline	ISO (5mg.kg^{-1})	ISO (25mg.kg^{-1})	Propranolol
H1	30.13	23.24	23.15	30.80
	22.92	32.83	21.89	22.17
	20.51	20.93	45.86	22.05
	$\bar{x}$ = 24.52 ± 2.89	$\bar{x}$ = 25.67 ± 3.64	$\bar{x}$ = 30.30 ± 7.79	$\bar{x}$ = 25.01 ± 2.90
H2	24.05	20.60	26.76	28.59
	37.09	29.97	29.26	25.21
	20.23	40.37	29.45	21.65
	$\bar{x}$ = 27.12 ± 5.10	$\bar{x}$ = 30.31 ± 6.71	$\bar{x}$ = 28.49 ± 0.87	$\bar{x}$ = 25.15 ± 2.00
H3	64.31	57.95	45.00	54.75
	56.03	42.76	45.19	47.04
	63.86	58.51	42.20	39.28
	$\bar{x}$ = 61.40 ± 2.69	$\bar{x}$ = 53.07 ± 5.16	$\bar{x}$ = 44.13 ± 0.97	$\bar{x}$ = 47.02 ± 4.47
H4	33.52	36.22	47.91	37.53
	33.31	38.97	36.99	31.73
	39.93	35.84	35.78	32.19
	$\bar{x}$ = 35.59 ± 2.17	$\bar{x}$ = 37.01 ± 0.99	$\bar{x}$ = 40.23 ± 3.86	$\bar{x}$ = 33.82 ± 1.86

* Mean values are ± S.E.M.

APPENDIX H

RESULTS OF STATISTICAL ANALYSIS

Effect of ISO Treatment Time on Histone Acetylation

	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
t	0.316	-0.056	1.629
D.F.	4	4	4
P	>.05	>.05	>.05
U	0.70	1.00	0.20

Effect of ISO Treatment Time on Histone Protein Yields

	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
t	-0.468	-0.740	0.209
D.F.	4	4	4
P	>.05	>.05	>.05
U	0.70	1.00	1.00

Effect of ISO Treatment Time on Acetylation in Histone Fractions

	1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
t	-0.419	-0.641	-0.360
D.F.	4	4	4
P	>.05	>.05	>.05
U	1.00	1.00	1.00

Effect of ISO Treatment Time on the Acetylation of Electrophoretically Purified Histones

Fraction		1 Hr. ISO	2 Hr. ISO	3 Hr. ISO
H1	t	-0.466	-0.614	-0.850
	D.F.	4	4	4
	P	>.05	>.05	>.05
	U	1.00	1.00	1.00
H2	t	-0.409	-0.560	-0.114
	D.F.	4	4	4
	P	>.05	>.05	>.05
	U	1.00	1.00	0.70
H3	t	-0.465	-0.645	-0.365
	D.F.	4	4	4
	P	>.05	>.05	>.05
	U	1.00	1.00	1.00
H4	t	-0.295	-0.757	-0.220
	D.F.	4	4	4
	P	>.05	>.05	>.05
	U	1.00	1.00	1.00

Effect of Perfusion Time on Histone Acetylation

Perfusion Time (Minutes)	t	D.F.	P	U
5	2.013	4	>.05	0.20
10	2.265	4	<.05	0.20
20	1.190	4	>.05	0.40
40	0.515	4	>.05	1.00
80	-0.716	4	>.05	0.70

Effect of Perfusion Time on Histone Protein Yields

Perfusion Time (Minutes)	t	D.F.	P	U
5	5.525	4	<.05	0.20
10	2.439	4	<.05	0.20
20	1.637	4	>.05	0.20
40	0.561	4	>.05	1.00
80	-0.262	4	>.05	1.00

Effect of Perfusion Time on the Acetylation of Electrophoretically Purified Histones

Perfusion Time (Minutes)	t	D.F.	P	U
5	0.216	4	>.05	0.70
10	0.463	4	>.05	1.00
20	0.800	4	>.05	0.70
40	0.299	4	>.05	1.00
80	3.074	4	<.05	0.20

Effect of Perfusion Time on Acetylation in Histone Fractions

Fraction	Perfusion Time (Minutes)	t	D.F.	P	U
H1	5	0.302	4	>.05	0.70
	10	-1.814	4	>.05	0.20
	20	-0.247	4	>.05	0.70
	40	-1.975	4	>.05	0.20
	80	0.650	4	>.05	1.00
H2	5	-0.732	4	>.05	0.70
	10	0.122	4	>.05	1.00
	20	-0.064	4	>.05	1.00
	40	0.493	4	>.05	1.00
	80	3.327	4	<.05	0.20
H3	5	0.705	4	>.05	0.40
	10	0.997	4	>.05	0.70
	20	1.431	4	>.05	0.40
	40	0.922	4	>.05	0.70
	80	-1.197	4	>.05	0.70
H4	5	-0.003	4	>.05	1.00
	10	1.172	4	>.05	0.40
	20	-0.597	4	>.05	0.70
	40	2.369	4	<.05	0.20
	80	1.569	4	>.05	0.40

Effect of 2 Hour Treatments with Saline, ISO (5mg.kg^{-1} BODY WEIGHT), ISO (25mg.kg^{-1} BODY WEIGHT), and Propranolol (25mg.kg^{-1} BODY WEIGHT) on Acetylation in Histone Fractions

Fraction	Treatment	t	D.F.	P	U
H1	ISO (5)	-0.247	4	>.05	0.70
	ISO (25)	-0.696	4	>.05	0.70
	Propranolol	-0.119	4	>.05	1.00
H2	ISO (5)	-0.417	4	>.05	0.70
	ISO (25)	-0.264	4	>.05	0.70
	Propranolol	0.360	4	>.05	1.00
H3	ISO (5)	1.431	4	>.05	0.40
	ISO (25)	6.046	4	<.05	0.20
	Propranolol	2.758	4	<.05	0.20
H4	ISO (5)	-0.597	4	>.05	0.70
	ISO (25)	-1.048	4	>.05	0.40
	Propranolol	0.619	4	>.05	0.40

VITA AUCTORIS

NAME: Donna Lynn Wakeman Young

BIRTH DATE: June 30th, 1955

EDUCATIONAL BACKGROUND:

1970 - 1973
Sisler High School
Winnipeg, Manitoba
Diploma: Grade 12

1973 – 1976
University of Manitoba
Faculty of Physical Education
Degree: Bachelor of Health, Physical Education, and Recreation

1977 - 1981
Univeristy of Windsor
Windsor, Ontario
Faculty of Human Kinetics
Degree: Master of Human Kinetics